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Award Number: DAMD17-99-1-9046

TITLE: Amplification of the Endothelin A Receptor Gene: A Potential Molecular Biomarker of Aggressive Prostate Cancer in African Americans

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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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Joel Nelson, M.D. 5/4/00

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13. ABSTRACT *(Maximum 200 words)*

Observations in our laboratory support the hypothesis that the endothelin axis is important in prostate carcinogenesis and progression, and that this pathway may be uniquely active in African American (AA) men, by virtue of potential amplification and overexpression of the endothelin receptor subtype A (ET_A). It is our hypothesis that the ET_A receptor is amplified in this patient population, and this contributes to a more aggressive disease course. In preliminary studies, Southern blot analysis of tumor DNA obtained from 5 African-American individuals was performed, using an ET_A cDNA probe: in every case, concentrations of ET_A DNA were greater in the tumor tissue (lymph node metastasis) compared to the corresponding normal tissue. The aims of this study were to determine whether the ET_A gene is amplified and differentially expressed in PCA from AA men, and if increased ET_A expression results in a more aggressive clinical course.

We found evidence for increased ET_A DNA in 20% of the samples studied, but we did not find convincing evidence for increased ET_A protein expression, using two complementary techniques. It is certainly possible a larger data set would include clear cases of ET_A overexpression at the DNA, RNA and protein levels.

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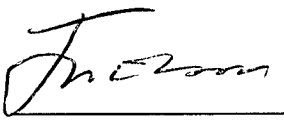

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Table of Contents

Introduction:

Observations in our laboratory support the hypothesis that the endothelin axis is important in prostate carcinogenesis and progression, and that this pathway may be uniquely active in African American (AA) men, by virtue of potential amplification and overexpression of the endothelin receptor subtype A (ET_A). It is our hypothesis that the ET_A receptor is amplified in this patient population, and this contributes to a more aggressive disease course. In preliminary studies, Southern blot analysis of tumor DNA obtained from 5 African-American individuals was performed, using an ET_A cDNA probe: in every case, concentrations of ET_A DNA were greater in the tumor tissue (lymph node metastasis) compared to the corresponding normal tissue. The aims of this study were to determine whether the ET_A gene is amplified and differentially expressed in PCA from AA men, and if increased ET_A expression results in a more aggressive clinical course.

Body:

Phase 1: Acquisition of Samples and Reagents (month 1)

In the initial phase of this project, we identified 10 normal/tumor pair samples from the prostate cancer tissue bank at the Brady Urological Institute for analysis. It was decided to first study a rather small and manageable set of samples to work out the details of the proposed analysis. Frozen sections (n=30) were obtained from each sample for immunohistochemistry and autoradiography. Additional sections were cut from the frozen tissue for DNA isolation. Genomic DNA was extracted using standard techniques. It was decided to not purchase an ET_A BAC clone until a tumor demonstrating ET_A amplification and overexpression was detected as a positive control.

Phase 2: Preliminary Experimentation Studying each tissue sample at multiple levels for ET_A expression (month 2-5)

As stated above, FISH analysis was not to be performed until a positive control, an ET_A over expressing tumor, could be identified.

Southern blot analysis was performed using the isolated genomic DNA extracted from normal/tumor pairs, digested with MspI, equally loaded and resolved on agarose gels. Following transfer to membranes, the blots were probed with an ET_A cDNA probe. We found evidence of increase ET_A DNA in two of the samples compared to their corresponding controls.

In stead of performing in situ hybridization, we acquired an ET_A-specific rabbit polyclonal antibody which was applied to each of the normal tumor pairs. In parallel to the ET_A staining, we studied expression of the other endothelin receptor, ET_B. Negative controls included omission of the antibody, nonspecific rabbit IgG, and preimmune rabbit serum. Positive controls included the media of blood vessels. As expected, we found expression of ET_A predominantly in the stromal component of the prostate gland with little to no expression in most of the benign epithelium. Areas of prostate cancer were also generally negative, although some ET_A staining was observed in areas of prostatic intra epithelial neoplasia (PIN). Interestingly, higher ET_B was noted in basal cells lining benign glands, but ET_B expression was lost in prostate cancer. Unfortunately, we also observed positive staining using preimmune rabbit serum in many of the areas read as positive (in the stromal component). At the time of this writing, we are adjusting antibody concentrations to limit this background.

On each section, autoradiography was also performed. Several attempts were required to obtain autoradiographs directly in the slides which were also stained with haematoxylon. Frozen

sections (20 μM) are incubated in one of the following solutions: total ET-1 binding (0.1 nM. ^{125}I-ET-1(label)), nonspecific binding (label + 1 μM. ET-1), total ET_B binding (label + 1 μM. BQ-123 or A-127722) and total ET_A binding (label + 0.1 μM. Sarafotoxin S6C or 1 μM. BQ-788). Rather than use the low resolution method previously described, we decided to use a high resolution emulsion technique. Similar to the findings using immunohistochemistry, we found the highest ET_A binding in areas of stroma: blood vessels provided a reliable internal control. We also observed ET_A binding in areas resembling PIN. We did not find increased ET_A binding in areas of infiltrating prostate cancer, even in the samples with increased ET_A DNA from the Southern blot analysis.

In summary, although we found evidence for increased ET_A DNA in 20% of the samples studied, we did not find convincing evidence for increased ET_A protein expression, using two complementary techniques. It is certainly possible a larger data set would include clear cases of ET_A overexpression at the DNA, RNA and protein levels. We found the amount of work required to fully analyze these 10 samples took nearly the entire time allotted. Despite an absence of data to support the initial hypothesis, we believe further investigation of ET_A expression in prostate cancer is warranted.

Phase 3: Data Analysis and Development of Grant Proposal Aims and Experiments (Month 6)

During the performance of this 6 month project, the DOD announced and submission was required for proposals directed towards prostate cancer. Given our preliminary data at the time of that request, we submitted a proposal to the DOD requesting additional time and funding to complete, in part, the studies outlined in the career development award. At the time of this

writing, the funding status of that proposal is unknown.

Key Research Accomplishments

- Increased ET_A DNA was observed in 20% of normal/tumor samples studied
- There was not a corresponding increase in ET_A protein expression in prostate cancer using immunohistochemistry and autoradiography
- ET_A protein expression was increased in areas of PIN

Reportable Outcomes

The only reportable outcome (drawing from the list provided) is the submission of a proposal (to the DOD) to study this question in more depth, with more samples, and over a longer time period.

Conclusions

We found evidence for increased ET_A DNA in 20% of the samples studied, we did not find convincing evidence for increased ET_A protein expression, using two complementary techniques. It is certainly possible a larger data set would include clear cases of ET_A overexpression at the DNA, RNA and protein levels. We found the amount of work required to fully analyze these 10 samples took nearly the entire time allotted. Despite an absence of data to support the initial hypothesis, we believe further investigation of ET_A expression in prostate cancer is warranted. Finally, the magnitude of the proposed project certainly exceeded the time allotted to such an

undertaking: put bluntly, in hindsight, this project could not have been performed in 6 months and requires a longer commitment of time and funding.

References

None

Appendices

None

Final Reports

There are no publications or meeting abstracts resulting from this preliminary project from any of the personnel receiving pay from the research effort (Joel B. Nelson, William Isaacs, Hong Zheng).

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September 21, 1999

Commander
U.S. Army Medical Research and Materiel Command
ATTN: MCMR-RMI-S
504 Scott Street
Fort Detrick, MD 21702-5012

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Thank you for your patience and support.

Sincerely,

Joel B. Nelson, M.D.

Enclosures

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
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Received 7/24/2001

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FOR THE COMMANDER:

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

Encl